Distribution and bioaccumulation of endocrine disrupting chemicals in water, sediment and fishes in a shallow Chinese freshwater lake: Implications for ecological and human health risks

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\text{ABSTRACT}

The occurrence, distribution and bioaccumulation of six endocrine disrupting compounds (EDCs) were investigated in water, sediment and biota samples from Luoma Lake, a shallow Chinese freshwater lake. Total concentrations of Ephiolnic EDGs were much higher than Zestroges EDGs in both waters and sediments. There were no obvious differences on the concentrations of target compounds [except nonylphenol (NP)] in upstream, lake and downstream locations, these may be suggested that they were mainly affected by non-point discharges in this area. However, the high concentration of NP in water may be associated with the discharge of rural domestic wastewater without thorough treatment. Furthermore, concentrations of NP were about 2–3 order magnitude higher than those of OP in both water and sediment compartments. Relatively higher bioaccumulation factors (BAF) were obtained for DES and EE2. Ecological risk assessment revealed greater risk of NP in surface water, which may pose a serious threat to aquatic ecosystems. The estrogen equivalent concentration (EEQ) of male were higher than those in female, and occurred in the order of city > rural-urban > countryside.

1. Introduction

Endocrine disrupting chemicals (EDC) are defined as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process (Diamanti-Kandarakis et al., 2009).” With progress of research on these chemicals, they are classified into natural and synthetic chemicals (Gorga et al., 2015). In 2013, the publications in World Health Organization (WHO) and United Nations Environment Programme (UNEP) concluded that EDGs can induce endocrine disorders of wildlife and humans (WHO, 2012). In fall 2015, Second Scientific Statement on EDC (EDC-2) drew conclusions about the strength of evidence between EDC exposures and obesity, diabetes and cardiovascular disease; female reproductive disorders; male reproductive disorders; hormone-sensitive cancers; thyroid conditions; and neurodevelopment and neuroendocrine effects (Gore et al., 2015).

EDGs have attracted global attention, because they can enter the most vulnerable ecosystem, aquatic environment, by different routes, including direct discharge of industrial and domestic wastewaters, discharge of wastewater treatment plants (WWTPs) effluents, agricultural drains to streams and rivers and overland flow after rainfall events (Gorga et al., 2015; Liao and Kannan, 2014; Lu et al., 2015; McAvoy et al., 2015; Ying et al., 2002; Zhang et al., 2015). Among the natural estrogens, 17β-estradiol (E2) is the most powerful estrogenic hormone and plays a major role in the complex mechanism of ovarian cycle in female (Guo et al., 2015; Rao et al., 2015). It excreted by both humans and livestock and deposited into river systems via WWTPs effluents (Zhang et al., 2008). The most potent man-made EDCs are 17α-ethynylestradiol (EE2) and diethylstilbestrol (DES). EE2 is an orally bioactive estrogen, and is one of the most commonly used medications for humans as well as livestock and aquaculture activity (Aris et al., 2014). It has become a widespread problem in the environment due to its high estrogenic potency (Esteban et al., 2014a). DES is a drug used in treating female canine incontinence stemming from poor sphincter control (Zhang et al., 2014). In addition, phenolic EDCs, including bisphenol A (BPA), nonylphenol (NP), and 4-tert-octylphenol (OP), are used in commercial products and industrial goods, such as thermal
samples (one sample per point) were collected in Luoma Lake with a water depth of 3.3 m and a surface water area of 260 km². The lake is located on the east part of the South-to-North Water Diversion Project (SNWDP), which is designed to ease water challenges in Northern China and thus contribute to socioeconomic development (Ren et al., 2007, 2015). Therefore, Luoma Lake is water diversion project with the main function of retain the water, and with comprehensive functions like water supply, aquaculture, irrigation, navigation, and tourism. Many villages and several factories were located in Fangting River, Yi River and Luoma lake flow major regions, a large quantity of living sewage water and industrial waste water enter this river by direct or indirect emission, which have become the main potential sources of EDCs in Luoma Lake (Shen et al., 2013; Zhang and Cui, 2012). Polycyclic aromatic hydrocarbons (PAHs) and heavy metal contaminations were previously studied in the sediments of this area (Chen et al., 2013; Liu et al., 2012). However, few studies, to our knowledge have investigated the distribution of EDCs in Luoma Lake. Thus, it is necessary to determine the concentrations of EDCs in aquatic environment which can provide scientific-based guidance for the management of risk. The objectives of this study were (1) to clarify the occurrence and distribution of EDCs (E2, E2E, DES, BPA, NP and OP) in surface water, sediment, and biota of Luoma Lake; (2) to estimate the bioaccumulation of these chemicals in biota, and (3) to assess their potential the ecological risk and health in the lake.

2. Materials and methods

2.1. Sample collection

A total of twenty-two water samples (100–150 cm) (one sample per point) were taken from Loma Lake region during April of 2016. At fourteen sampling points located in the Loma Lake, four samples were collected in two inflowing rivers (Yi River; Fangting River), the other four points corresponded to the outflowing rivers (south of Zhongyun River; Xinyi River, through Zhangshan Lock) (Fig. 1). Water samples were preserved in 2 L brown glass containers bottles that had been precleaned and transported to the laboratory, where they were filtered immediately through 0.45 µm × 50 mm glass fiber filters (0.45 µm). pH, temperature (T), water transparency (WT) and dissolved oxygen (DO) of water samples were shown in Table S1 (supplementary material). There are many sands and gravels in the riverbed, only six sediment samples (one sample per point) were collected in Luoma Lake with a Peterson grab sampler. The sediments were wrapped into stainless steel containers, freeze dried, ground, sieved and stored at −20 °C for further extraction. Two dominant fish species (Grass Carp and Lateolabrax japonicus) were bought immediately after they were caught from local fishers. Biota samples were immediately placed in coolers with ice, transported to the laboratory freeze-dried and stored at −20 °C for further extraction.

2.2. Reagents and standards

Pure standards of the target compounds E2, EE2, DES, BPA, NP and OP were purchased from J & K Chemical, Ltd. (St. Louis, MO, USA). Detailed substance information for all the target compounds is summarized in Table S2. Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Ammonia (25%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All organic solvents used in this study were HPLC grade. Deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Stock solutions of all target chemicals at concentrations of 1 g/L were prepared in methanol were stored at 4 °C.

2.3. Sample treatment and analyses

The method of pretreatment for water samples was based on our previous studies (Ding et al., 2014). Briefly, water samples (1 L) were extracted by solid-phase extraction (SPE). All Oasis HLB cartridges (500 mg, 6 mL, Waters) were conditioned with 5 mL methanol and 5 mL ultrapure water. Filtrates were then passed through the cartridges at a flow rate of about 5 mL/min. Following extraction, the analytes were eluted with 10 mL methanol after drying for 30 min, and the solution was then concentrated to 1 mL under a gentle stream of N2.

Ten gram of each sediment samples were extracted by ultrasonic extraction and amened with a mixture solvent of 40 mL hexane-chloromethane (v/v = 1:4), then extracted for 30 min and centrifuged at 8000 rpm for 5 min. The extract was then added into a flask and concentrated to dry, after which it was redissolved into 1 mL of methanol. Next, 99 mL of ultrapure water were added to the solution for further cleanup by SPE. The procedures were similar to that in water samples.

The fish muscle was collected after removing internal organs, bones and skin. Only fish muscle was collected for chemical analysis. Ten gram of homogenized fish samples were stored in glass bottles, mixed with anhydrous sodium sulfate and then extracted by ultrasonic extraction and amened with a mixture solvent of methanol-acetone (v/v = 1:1). The extraction was repeated twice with total 25 mL solvent and then combined the extract. The extract was then added into a flask and concentrated to almost 0.5 mL. Next, 100 mL of ultrapure water were added to the solution for further cleanup by SPE. The procedures were similar to that in water samples.

The extracts were finally analyzed by HPLC-MS/MS (LC-Agilent Technologies 1290 Infinity, MS-AB SCIEX QTRAP 4500; CA), after which chromatographic analysis was performed with a ZORBAX Eclipse Plus C18 column (2.1 mm × 150 mm, 3.5 µm; Agilent). The column was maintained at 30 °C, and the injection volume was 2 µL. Gradient elution programs are presented in Table S3A. The mass spectrometer was operated in negative electrospay ionization and in multiple reaction monitoring mode. The typical mass spectrometric conditions are provided in Table S3B.

2.4. Quality assurance and quality control

To ensure reliable results, a strict protocol was established. In all sample preparation procedures (including sample collection), plastic material and detergents were discarded to avoid contamination and blank problems. Furthermore, glassware was carefully washed with acetone, Milli-Qwater and methanol prior to use. The analytical method was evaluated under optimized conditions in terms of linearity, relative standard deviations, method quantification limits (MQLs), and recoveries. For water, sediment and biota samples, relative recoveries varied from 74.6% to 101.3% (RSD < 3%), from 74.0% to 101.2% (RSD < 3%) and from 72.8% to 87.4% (RSD < 6%), respectively. The MQLs for each compound ranged from 2 to 5 ng/L in water, 0.2–1.0 ng/g in sediments, and 0.2–1.0 ng/g in biota. Detailed method performance parameters are summarized in Table S4.

2.5. Statistical analyses

The correlation coefficient between the target EDCs were evaluated by Pearson’s test. Linear regression was used to evaluate the relationship of the individual EDCs concentrations in water and sediment. The
level of statistical significance was defined at $p < 0.05$. All statistical analyses were carried out with SPSS 11.5 for Windows (SPSS, Inc., Chicago, IL).

3. Results and discussion

3.1. EDCs in surface water

The concentrations of the target EDCs in surface water in Luoma Lake are shown in Fig. 2 and Table S5. E2, EE2, BPA and OP were detected in all 22 water samples, and the detection frequency of NP was 63.6%, whereas DES was only detected in FTR2 (5.65 ng/L). The concentration of natural estrogens (E2) ranged from 2.52 ng/L to 21.82 ng/L (average concentration 9.41 ng/L). The total concentrations of the synthetic estrogens (EE2) ranged from 4.25 ng/L to 2.85 ng/L (average concentration 7.97 ng/L). The total concentrations of the rest three phenolic EDCs (BPA, NP and OP) ranged from nd (not detected) to 1758.40 ng/L (average concentration 49.38 ng/L, 769.96 ng/L and 4.67 ng/L, respectively). Apparently NP was the most abundant EDCs among the measured chemicals, remaining EDCs decreased in the order of BPA > E2 > EE2 > OP > DES in the surface water. The high detection level of NP could be attributed to its frequent use in China, with an annual production of more than 30,000 t in 2011 in China, according to the record of China Petroleum and Chemical Industry Federation (CPCIF).

The total concentrations of target compounds at different positions differed greatly. Concentrations of EDCs were clearly higher at FTR2, ZSL01, and LML06, and the maximum EDCs concentration occurred at inflowing rivers of FTR2 (1857.01 ng/L), while the minimum concentration occurred at Luoma Lake of LML10 (93.68 ng/L). The total concentrations of target compounds (except NP) at each point ranged from 73.00 to 158.36 ng/L. In addition, there were not significant differences between the concentrations of target compounds in upstream locations (FTR1, FTR2, YR1 and YR2) with those in the lake and downstream locations (ZYR1, ZYR2, ZSL1 and ZSL2), indicating a strong influence of non-point discharges in this area. It was notable that NP was dramatically different in all sampling sites, which may be attributed to the point sources nearby. The lake is surrounded by densely populated residential area; therefore, a large amount of rural domestic wastewater without thorough treatment was discharged into Luoma Lake, as NP was mainly used in the productions of surfactant in household goods, suggesting that they probably contributed an important source of NP in this lake (Fan et al., 2013; Shen et al., 2013; USEPA, 2010; Zhang and Cui, 2012). Furthermore, there are also some chemical enterprises, dyeing facilities and paper manufacturers located along the both banks of the lake, part of the industrial wastewater can enters the lake from wastewater treatment plant (WWTPs) (Fan et al., 2013). Interestingly, the concentrations of NP (nd-1758.40 ng/L) were in many sampling sites basely 2–3 order magnitude higher than those of OP (4.17–5.64 ng/L). The finding was similar to the relationship between NP and OP in surface water of other countries (Liu et al., 2017; Watanabe et al., 2007; Zhang et al., 2009). This is probably due to the lower hydrophobicity of OP (log Kow=5.5) than NP (log Kow=5.99) (EPI Suit (Isobe et al., 2001). Furthermore, NP and OP are the main metabolism products of nonylphenol polyethoxylates (NPEOs) and octylphenol polyethoxylates (OPEOs) respectively account for about 80% and 20% of total APEOs which have been widely used in household, agriculture and industrial processes (Chiu et al., 2010; Esteban et al., 2014b; Morales et al., 2009; Sharma et al., 2009).

These results were compared with the published results and
presented in Table S6. Concentrations of estrogens in this study are completely different with them in other studies, especially in the case of Thailand rivers where E2 (1400 ng/L) and EE2 (360 ng/L) were found at about 1–2 orders of magnitude higher concentrations, which may be contributed by the discharge from WWTPs (Ruchiratset and Chinwetkitvanich, 2014). In surface waters of some countries, such as Turkey, Malaysia and Spain, concentrations of E2 was similarly low than in Luoma Lake (Esteban et al., 2014b; Oguz and Kankaya, 2013; Praveena et al., 2016). Concentrations of EE2 in the Taihu Lake and river in Malaysia were nd−33.50 ng/L and nd−0.005 ng/L, respectively, and even not detected in Songhua River, China (Praveena et al., 2016; Wang et al., 2015; Zhang et al., 2014). However, concentrations of DES were similar to the concentrations found in the Songhua River (nd−1.38 ng/L). For phenolic EDCs, concentrations detected in this study were higher than those reported for the Panlong river of Yunnan-Guizhou plateau in China (Wang et al., 2016). The average concentrations of BPA (92.57 ng/L) in Taihu Lake were close to the concentration of BPA found in the Luoma Lake (86.41 ng/L) (Liu et al., 2016). Lower concentrations than those reported in this study were found in other Chinese rivers located in Wuhan (nd−37.1 ng/L), Yunnan-Guizhou (12−79 ng/L) and Yangtze Estuary (LOQ−2.0 ng/L) (Shi et al., 2014; Wang et al., 2016; Wu et al., 2015). OP in Songhua River of China, river in Central Spain, and Minho River in European has previously been shown at concentrations ranging from 1.54 to 45.8 ng/L, 0.14−474 ng/L and 8−88 ng/L, respectively, which was much higher than the concentrations observed in the present study (4.17–5.64 ng/L) (Esteban et al., 2014b; Salgueiro-González et al., 2015; Zhang et al., 2014). In addition, Esteban and Lu found that NP in surface water of Taihu Lake (262.39–1442.72 ng/L) and Spain (96–1483 ng/L) was comparable to that in Luoma Lake (nd−1758.4 ng/L) (Esteban et al., 2014b; Lu et al., 2011).

3.2. EDCs in sediment

The sediment samples in Luoma Lake were analyzed to determine occurrence and distribution of the target contaminants. Only six samples were collected which sited in LMR01, LMR03, LMR09, LMR10, LMR11 and LMR12, respectively. The concentrations of the contaminants in sediment of Luoma Lake are shown in Fig. 3 and Table S7. The total concentrations of target compounds ranged from 46.48 to 57.59 ng/g dw. They were detected in all sediment samples, except of DES, with the detection frequencies of 33% which was similar to the low concentration and low detection frequency observed for waters. The concentration of natural estrogens (E2) ranged from 0.52 to 1.21 ng/g dw (average concentration 0.85 ng/g dw). The total concentrations of two synthetic estrogens (EE2 and DES) ranged from 0.61 to 1.48 ng/g and nd−0.56 ng/g (average concentration of 0.87 ng/g and 0.18 ng/g, respectively. Concentrations of three phenolic EDCs varied between 0.43 and 47.04 ng/g dw, with a major contribution of NP (80% to the total EDC concentrations) which demonstrated the high capability of this compound to be accumulated in sediments, the same conclusion can also be drawn from previous studies (Salgueiro-González et al., 2015). The concentrations of target compounds ranged from 46.48 to 57.59 ng/g dw at all sampling sites. As noted before, concentrations in sediments showed the similar trend with those in water samples, as well, which could be associated with the deposition of suspended particulate matter from water compartment. The correlations (r=0.943, p < 0.01) of each individual compound in water and sediment compartment indicated strong and significantly positive relationships. A reasonable explanation for this is that the deposition and resuspension of suspended particulate matter plays an important role in these two compartments. It was notable that the concentrations of NP in sediment were also basely 2 order magnitude higher than those of OP, consistent with the observation in surface water.

By analyzing in detail the available data it can be seen that the levels of E2 detected in this study are in the same order as in Songhua River (nd−1.16 ng/g dw) and Pearl River Delta 0.9−2.6 ng/g dw) in China and much lower than levels reported in Taihu Lake of China (Zhang et al., 2014). Wang, et al. reported much higher concentration of EE2 in Taihu Lake of China which was explained by the discharge of a large amount of effluent from WWTPs as well as untreated domestic sewage. Zhang, et al. found that DES in Songhua River of China (nd−0.4 ng/g dw) was comparable to it in this study (Zhang et al., 2014). In addition, concentrations of three phenolic EDCs (BPA, NP and OP) in sediments of the Taihu Lake were nd−99.20, nd−65.08 and 1.1−209.8 ng/g dw, respectively, which were higher than these containments in Luoma Lake (Liu et al., 2016). The same tendency of BPA in sediments of Panlong River 6.6−124 ng/g dw) and Pearl River Delta (1.7−430 ng/g dw) in China and European river basin (4.3−130 ng/g dw)(Gong et al., 2011; Salgueiro-González et al., 2015; Wang et al., 2016). The concentrations of NP in this study were much lower than in the Pearl River Delta in China and Minho River estuary in European, higher than than in the Songhua River and higher than sediments that in Panlong river (Gong et al., 2011; Salgueiro-González et al., 2015; Zhang et al., 2014). Compared with other available investigations on estrogens that in Songhua River, the results of the present study were slightly higher (Zhang et al., 2014) (Fig. 3).

3.3. EDCs in biota

The concentrations of EDCs in aquatic organism samples from Luoma Lake are presented in Fig. 4. E2, EE2, BPA, NP and OP were detected in both of two types of fish species, while DES was only detected in Grass Carp. Concentrations of all estrogens (E2, EE2 and DES) and phenolic EDCs (BPA) detected in Grass Carp were higher than these in Lateolabrax japonicus. In contrast to BPA, NP and OP in Lateolabrax japonicus were about twice as much as in Grass Carp. In general, the average concentrations of EDCs (E2, EE2, DES, BPA, NP and OP) in fishes were 0.85, 1.18, 0.11, 7.56, 232.30 and 0.66 ng/g dw, respectively. It is essential to point out that E2 can be present naturally in aquatic organisms, especially in fish (Xie et al., 2015). Unfortunately, endogenous E2 was not distinguished from the detected concentrations in the present study. E2 was previously detected in crucian carp and carp from laboratory and field control groups with the average concentrations ranging from non-detection (< 0.6 ng/g dw) to 1.6 ng/g dw in muscle samples (Huang et al., 2013; Liu et al., 2012b).

In comparison with these previous observations, the same range concentrations of E2 (0.58−1.87 ng/g dw) were found in Luoma lake. This suggested E2 being detected may came from organism itself. Nevertheless, more complete experimental design, such as laboratory control and field control, should be employed to determine endogenous
E2 in fish in future work. The occurrence of estrogens has been rarely reported in aquatic organisms around the world. The concentrations of E2 and EE2 in fish from Taihu Lake (maximum concentrations of 562 and 417 ng/g dw) (Wang et al., 2015; Xie et al., 2015) were much higher than those in this study, and were in the same range as in St. Clair River of Canada (EE2: mean concentration of 1.52 ng/g, ww) and Dianchi Lake, China (ranging from nd-4.8 and nd-11.3 ng/g dw) (Al-Ansari et al., 2010; Liu et al., 2011). There are many studies in the literature on the status of phenolic EDCs in biota. The concentrations of BPA, NP, and OP in fish species in Panlong river (ranging from 1.9 to 69, nd-19.0, and nd-4.4 ng/g dw, respectively) and Dianchi Lake (ranging from 10.1 to 83.5, nd-18.9, and nd-4.6 ng/g dw, respectively) were much higher than those in Luoma Lake (ranging from 5.22 to 13.40, 164.2-300.63, and 0.38-0.96 ng/g dw, respectively), and were higher than Lake Skinner in California (5.06-8.94 ng/g, dw, nd and nd), expect NP (Liu et al., 2011; Wang et al., 2016; Yu and Wu, 2014). The highest concentrations of BPA (31-412 ng/g, dw), NP (306-1756 ng/g, dw), and OP (19.9-39.7 ng/g, dw) were found in Corbicula fluminea from European river basin (Salgueiro-González et al., 2015). These differences may attribute to the consumers class, body shape and dietary sources of the organisms.

3.4. Estimation of bioaccumulation of the target compounds

Bioaccumulation factor (BAF) is commonly used metrics in risk assessment to predict bioaccumulation of chemical contaminants in aquatic organisms. According to the United States Environmental Protection Agency (US-EPA) (Us Epa, 2000), BAF is defined as the ratio (in liters per kilogram of tissue) of the concentration of a chemical in the tissue of an aquatic organism to its concentration in water, in situations where both the organism and its food are exposed (Salgueiro-González et al., 2015). In our study, these parameters were estimated according to the following equations:

$$BAF = \frac{C_{biota}}{C_{water}}$$  

where $C_{biota}$ and $C_{water}$ were the average concentrations of the target EDCs measured in each fish and the surface water and sediment samples, respectively. On the basis of the average concentrations of target EDCs in the fish muscle and in water, and the field BAFs (expressed in L/kg) of E2, EE2, DES, BPA, NP and OP were calculated in two fish species as shown in Table 1. Chemicals with BAF values > 1000, 2000 or 5000 L/kg are considered bioaccumulative factors by various regulatory authorities (Klosterhaus et al., 2013). Relatively higher average BAFs were estimated for DES (884.21 L/kg) in Grass Carp and EE2 (325.20 L/kg) in Lateolabrax japonicus. BAFs for the target EDCs were lower than 1000 L/kg, suggesting that their low bioaccumulation potential in aquatic organisms from Luoma Lake. Compared with other EDCs in fish, the BAFs for E2, EE2 and BPA of Taihu Lake (697, 4115 and 1025 L/kg), NP in Taiwan (6400 L/kg) and OP European river basin (439 L/kg) were much higher than these in this study (Lee et al., 2015; Salgueiro-González et al., 2015; Wang et al., 2015). The reported values are much higher than these in Luoma Lake, but the comparison is not straightforward as the characteristics which could be explained by hydrophobic and metabolic characteristics, environmental characteristics (emission source, temperature, pH, etc.) as well as by organisms’ characteristics (lipid content, health status, and gender or life stage) which could affect the distribution of the compounds. Therefore, more studies about the bioaccumulation of EDCs in biota are needed (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
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<th>Endpoint</th>
<th>Value (μg/L)</th>
<th>AF</th>
<th>PNEC (ng/L)</th>
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<td>NOEC</td>
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<td>50</td>
<td>200</td>
<td>(Liu et al., 2012a)</td>
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<tr>
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<td>NOEC</td>
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<td>10</td>
<td>10</td>
<td>(Stange et al., 2012)</td>
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<tr>
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<td>Amphibians</td>
<td>NOEC</td>
<td>1</td>
<td>50</td>
<td>20</td>
<td>(Li, 2015)</td>
</tr>
</tbody>
</table>

3.5. Implications for ecological

3.5.1. Environmental risk assessment

The aquatic risk assessment for the measured EDCs was evaluated on the basis of risk quotient (RQ) estimation, which is the ratio between a measured environmental concentration (MEC) and a predicted no effect concentration (PNEC), as described in Eq. (1):

$$RQ = \frac{MEC}{PNEC}$$  

The PNEC value was obtained from the acute toxicity data (LC50 or EC50) divided by an assessment factor (AF) of 1000. Once the long-term/chronic NOEC values for one, two, or three trophic concentrations are available, an AF of 1000, 100, 50, or 10 is used (Directive, 2003). The toxicity data of multiple species used in this study were mainly collected from the literature and the USEPA ECOTOX database (Table 2) (http://cfpob.epa.gov/ecotox). For RQ estimation in this study, MEC measured in water samples were considered. Furthermore, the PNEC values of BPA, NP and OP were collected from our previous study (Liu et al., 2016). The risk was classified into three levels, i.e. low risk with the RQs ranging between 0.01–0.1, medium risk with the RQs ranging between 0.1–1, and high risk with the RQs > 1 (Hernando et al., 2006). The results were illustrated in Fig. 5. In general, the RQ values of all target compounds in surface water declined according to the following order: NP > EE2 > E2 > DES > BPA = OP. The RQ values of NP and EE2 exceeded 1 at some of the sampling sites. Furthermore, the maximum RQ value for NP at inflow river was more than 7, which indicated that aquatic organisms were exposed at a high risk and the upstream maybe an emission source. The other target compounds

![Fig. 4. Concentrations of EDCs in biota from Luoma Lake.](https://example.com/fig4.png)
 showed a medium risk to low risk at most sites.

### 3.5.2. Human health impact

Since EDCs exist in the aquatic environment in mixtures and act additively to induce similar estrogenic effects, it is necessary that the combined toxicity should be calculated for assessing the risk of an EDC to human health. Diet is considered to be among the main routes of EDC exposure (Geens et al., 2015). Taking into account that, as a worst case scenario, water and biota of Luoma Lake could be consumed by the population of this area, the possible health risk for the residents should be evaluated.

Calculation of the estrogen equivalent concentration (EEQ) of a chemically determined mixture is based on all measured xenoestrogens with a known estradiol equivalency factor (EEF) according to (Voogt et al., 2014):

\[
EEQ_i = C_i \times EEF_i, \quad \text{and} \quad EEQ = \sum EEQ_i
\]

where \( i \) refers to the compound \( i \) with concentration \( C \), \( EEQ_i \) is the total estradiol equivalent quantity and \( EEF \) is the estradiol equivalency factor. The EEFs were defined as the EC50 of each compound relative to the EC50 of 17β-estradiol. The EC50 is defined as the concentration of an agonist, which produces 50% of the maximum possible effective response for that agonist. In this study, literature EEF values of target EDCs were determined by estrogen receptors (ERs) α in vitro test (Zhang et al., 2011). For calculations, the estrogenic potential of each sample within the same sampling campaign were averaged. Table 3 shows the contribution of each compound to the total estrogenic activity.

As shown in Table 3, the concentrations of estrogenic compounds EEQ in surface water and biota were 19.27 ng/L and 2.46 ng/g, respectively. For the EEQ in water of this study, the main contributor to the estrogenic potential of waters were E2, which accounted by more than 49% of the total EEQs. Although NP was the predominant compound in surface water, with mean concentrations of 769.96 ng/L, it is the third least relevant compound in terms of estrogenic activity.

Regarding the biota samples, the main contributor to the estrogenic potential was EE2 which accounted by more than 55.6% of the total EEQs since the mean concentration of it was up to 1.37 ng/g in biota. Comparing the EEQ level of estrogenic compounds with other studies, the concentration of EEQ was comparable to it in Xiamen, China, where nearly 20 ng/L in surface water (Zhang et al., 2011). The concentration of EEQ was much higher than it in European countries, where the EEQ level was less than 3 ng/L in water (Salgueiro-González et al., 2015).

The effect of the human exposure to these EDCs via water ingestion and aquatic products intake and will be estimated by the data in Table 4 (Duan, 2013; MEP, 2015). Fig. 6 showed the levels of exposure via water ingestion and aquatic products intake in terms of EEQ for children (under 5 years old) and adults (male and female).

Finally, the sum estimated daily intake was compared with the acceptable daily intake (ADI) of E2 to evaluate the possible risk to human health. According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), ADI of E2 for a person is 0.05 μg/kg bw (JECFA, 1999). As shown in Fig. 6(a), there were two conclusions to be drawn as follows: 1. the concentration of EEQ in water were all higher than those in biota; 2. the EEQ level in older children were generally higher than young children. As shown in Fig. 6(b), it is worth noting that the concentrations of EEQ of male were higher than those in female, and the concentrations of EEQ occurred in the order of city > rural-urban > countryside. These findings showed the influencing factors included metabolic rate, labor intensity, consumption level and pollution levels. As a worst case scenario, the estimated results obtained in this study were much lower than the maximum allowable values, suggesting that no potential adverse effects of measured EDCs on local residents’ health through water ingestion and aquatic products intake.

### 4. Conclusions

Concentrations of three estrogens endocrine disrupting chemicals [17β-estradiol (E2), 17α-ethynylestradiol (EE2), diethylstilbestrol (DES)] and three phenolic estrogens [bisphenol A (BPA), nonylphenol (NP) and 4-tert-octylphenol (OP)] were determined in surface water, sediment and biota samples collected from Luoma Lake, the fourth largest freshwater lake in Jiangsu province, China.

The concentrations of the target EDCs in ranged from 93.68 ng/L to 1857.01 ng/L, decreased in the order of NP > BPA > E2 > EE2 > OP > DES in the surface water. The presence of NP in the lake could be mainly associated with the discharge of rural domestic wastewater without thorough treatment. Concentrations of EDCs in sediments may originate from the deposition of suspended particulate matter from water compartment. Concentrations of all estrogens (E2, EE2 and DES) and phenolic EDCs (BPA) detected in Grass Carp were higher than these in Lateolabrax japonicus. While, NP and OP in Lateolabrax japonicus were about twice as much as in Grass Carp.

Bioaccumulation factor were all below 1000 L/kg, suggesting their low bioaccumulation potential in aquatic organisms from Luoma Lake.

For the total estrogenic activity, main contributors to the estrogenic potential were E2 in water and were EE2 in biota. Furthermore, EEQs of male were higher than those in female, and the concentrations of EEQ

**Table 3**

<table>
<thead>
<tr>
<th>Compound</th>
<th>EEF</th>
<th>Cwater (ng/L)</th>
<th>EEQwater (ng/L)</th>
<th>%</th>
<th>Cbiota (ng/g)</th>
<th>EEQbiota (ng/g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>1.00</td>
<td>9.41</td>
<td>9.41</td>
<td>48.8</td>
<td>0.85</td>
<td>0.85</td>
<td>34.6</td>
</tr>
<tr>
<td>EE2</td>
<td>1.16</td>
<td>7.97</td>
<td>9.24</td>
<td>48.0</td>
<td>1.18</td>
<td>1.37</td>
<td>55.6</td>
</tr>
<tr>
<td>DES</td>
<td>1.75</td>
<td>0.26</td>
<td>0.46</td>
<td>2.4</td>
<td>0.11</td>
<td>0.20</td>
<td>8.1</td>
</tr>
<tr>
<td>BPA</td>
<td>2.30E–04</td>
<td>86.41</td>
<td>1.99E–02</td>
<td>0.1</td>
<td>7.56</td>
<td>1.74E–03</td>
<td>0.1</td>
</tr>
<tr>
<td>NP</td>
<td>1.75E–04</td>
<td>769.96</td>
<td>0.13</td>
<td>0.7</td>
<td>322.30</td>
<td>4.07E–02</td>
<td>1.7</td>
</tr>
<tr>
<td>OP</td>
<td>7.00E–04</td>
<td>4.67</td>
<td>3.27E–03</td>
<td>–</td>
<td>0.66</td>
<td>4.59E–04</td>
<td>–</td>
</tr>
<tr>
<td>EEQt</td>
<td>19.27</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 4

Body weight (kg), water ingestion (L/d) and aquatic products intake (kg/d) for Child and Adult in China.

<table>
<thead>
<tr>
<th>Index</th>
<th>0 Month−</th>
<th>3 Month−</th>
<th>6 Month−</th>
<th>9 Month−</th>
<th>1 Year−</th>
<th>2 Year−</th>
<th>3 Year−</th>
<th>4 Year−</th>
<th>5 Year−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>6.4</td>
<td>7.9</td>
<td>9.1</td>
<td>9.8</td>
<td>11.2</td>
<td>13.5</td>
<td>15.6</td>
<td>17.7</td>
<td>19.6</td>
</tr>
<tr>
<td>m-ADI (µg)</td>
<td>0.32</td>
<td>0.40</td>
<td>0.46</td>
<td>0.49</td>
<td>0.56</td>
<td>0.68</td>
<td>0.78</td>
<td>0.89</td>
<td>0.98</td>
</tr>
<tr>
<td>Water ingestion (L/d)</td>
<td>0.18</td>
<td>0.34</td>
<td>0.59</td>
<td>0.81</td>
<td>0.91</td>
<td>0.81</td>
<td>0.86</td>
<td>0.85</td>
<td>0.86</td>
</tr>
<tr>
<td>Aquatic products intake (kg/d) *</td>
<td>1.92E−04</td>
<td>2.37E−04</td>
<td>2.73E−04</td>
<td>2.94E−04</td>
<td>2.46E−03</td>
<td>2.97E−03</td>
<td>2.96E−03</td>
<td>3.36E−03</td>
<td>3.72E−03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Index</th>
<th>City</th>
<th>Rural-Urban</th>
<th>Countryside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>Mean</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>62.0</td>
<td>60.6</td>
<td>65.0</td>
<td>56.8</td>
</tr>
<tr>
<td>3.10</td>
<td>3.03</td>
<td>3.25</td>
<td>2.84</td>
</tr>
<tr>
<td>1.80</td>
<td>1.85</td>
<td>2.00</td>
<td>1.71</td>
</tr>
<tr>
<td>Aquatic products intake (kg/ d)</td>
<td>2.26E−02</td>
<td>1.39E−02</td>
<td>1.06E−02</td>
</tr>
</tbody>
</table>

occurred in the order of city > rural-urban > countryside. The estimation of daily intake of the target EDCs in Luoma Lake showed no risk to human health via water and biota ingestion.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2017.02.045.

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